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CARDIOVASCULAR

Do intravascular hypo- and hypervolaemia result in changes in central blood volumes?

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Abstract

Background: Hypovolaemia is generally believed to induce centralization of blood volume. Therefore, we evaluated whether induced hypo- and hypervolaemia result in changes in central blood volumes (pulmonary blood volume (PBV), intrathoracic blood volume (ITBV)) and we explored the effects on the distribution between these central blood volumes and circulating blood volume ($V_{d\text{ circ}}$).

Methods: Six anaesthetized, spontaneously breathing Foxhound dogs underwent random blood volume alterations in steps of 150 ml (mild) to 450 ml (moderate), either by haemorrhage, retransfusion of blood, or colloid infusion. PBV, ITBV and $V_{d\text{ circ}}$ were measured using (transpulmonary) dye dilution. The $PBV/V_{d\text{ circ}}$ ratio and the $ITBV/V_{d\text{ circ}}$ ratio were used as an assessment of blood volume distribution.

Results: 68 blood volume alterations resulted in changes in $V_{d\text{ circ}}$ ranging from –33 to +31%. PBV and ITBV decreased during mild and moderate haemorrhage, while during retransfusion, PBV and ITBV increased during moderate hypervolaemia only. The $PBV/V_{d\text{ circ}}$ ratio remained constant during all stages of hypo- and hypervolaemia (mean values between 0.20–0.22). This was also true for the $ITBV/V_{d\text{ circ}}$ ratio, which remained between 0.31 and 0.32, except for moderate hypervolaemia, where it increased slightly to 0.33 (0.02), $P < 0.05$.

Conclusions: Mild to moderate blood volume alterations result in changes of $V_{d\text{ circ}}$, PBV and ITBV. The ratio between the central blood volumes and $V_{d\text{ circ}}$ generally remained unaltered. Therefore, it could be suggested that in anaesthetized spontaneously breathing dogs, the cardiovascular system maintains the distribution of blood between central and circulating blood volume.

Key words: anaesthesia; blood volume; dogs; hypovolemia; indicator dilution

Circulation of blood is necessary for all mammals to maintain homeostasis. To accomplish this, the cardiovascular system is able to generate cardiac output by a complex interaction of multiple factors, of which venous return to the heart, the degree of vascular filling and cardiac performance are key factors.¹ The first two factors are particularly influenced by the individual's actual circulating volume. Whenever cardiac output is to be

optimized, an adequate assessment of volume status is crucial to prevent excessive morbidity and/or mortality associated with either inadvertent hypo- or hypervolaemia.^{2,3} While the traditionally used static pressure-based indicators of cardiac preload such as central venous pressure (CVP) or pulmonary capillary wedge pressure are relatively easy to measure, they have been shown to be inadequate in assessing cardiac preload.^{4,5} Instead, dynamic

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Editor's key points

- It is generally believed that the response to hypovolaemia includes redistribution of blood from peripheral to central vessels.
- In this animal study, induced hypovolaemia and hypovolaemia produced linear changes in central blood volumes.
- However, the ratios between calculated central and total circulating volumes were constant.
- These data challenge previous assumptions regarding redistribution of blood in response to hypovolaemia, but more studies are needed.

preload variables (e.g. pulse pressure variation and stroke volume variation) are more adequate in assessing fluid responsiveness, but unfortunately multiple factors, such as spontaneous breathing activity or cardiac arrhythmias, prohibit the clinical use of these variables in many clinical situations.⁶ Also, dynamic preload variables are a surrogate measure of fluid responsiveness and do not provide exact quantification of volume status. Instead, indicator-dilution techniques allow the determination of blood volumes, such as circulating blood volume ($V_{d\text{ circ}}$) and central blood volume compartments [i.e. pulmonary and intrathoracic blood volume (PBV and ITBV, respectively)].^{7,8}

Acute haemorrhage and (re)transfusion resulting in hypo- or hypervolaemia (i.e. an absolute change in $V_{d\text{ circ}}$) might subsequently affect the intrathoracic compartment (i.e. PBV or ITBV). In addition, inducing hypo- and hypervolaemia might also influence the distribution of blood between the intra- and extrathoracic blood volume compartments. For instance, in case of hypovolaemia, an individual is traditionally assumed to have a 'centralized' circulation with peripheral vasoconstriction, in order to assure adequate blood flow to vital organs and for preservation of venous return, which should be reflected by an altered distribution between $V_{d\text{ circ}}$ and PBV or ITBV, in favour of the central blood volumes. Previously, the cardiovascular response to fluid alterations (i.e. haemorrhage) has been assessed in experimental conditions in humans and in dogs.^{9–12} However, the effects of fluid alterations on the distribution of blood volume have to the best of our knowledge, not yet been investigated. Furthermore, alterations of fluid status occur most frequently while patients are under general anaesthesia, further prompting the necessity to evaluate the distribution of blood volume under such conditions, as general anaesthesia might influence the 'centralization' response.^{11,13}

Therefore, the aim of the present study was to determine $V_{d\text{ circ}}$ and central blood volumes (PBV, ITBV) in anaesthetized, spontaneously breathing dogs, during induced alterations of blood volume resulting in hypo- or hypervolaemia and subsequently calculating the relationship between circulating and central blood volumes. We hypothesized that the ratio between these central blood volumes and circulating blood volume is dependent on the actual volume status.

Methods

Animals and instrumentation

The reporting of this study was set up in accordance with the ARRIVE guidelines for reporting animal studies¹⁴ (online-only Supplementary data, Fig. S1). The dogs (six Foxhounds of both sexes (3 males, 3 females), body weight between 28 and 35 kg, mean (SD) 29 (3) kg; all healthy, not splenectomized) were treated according to the principles of the National Institute of Health

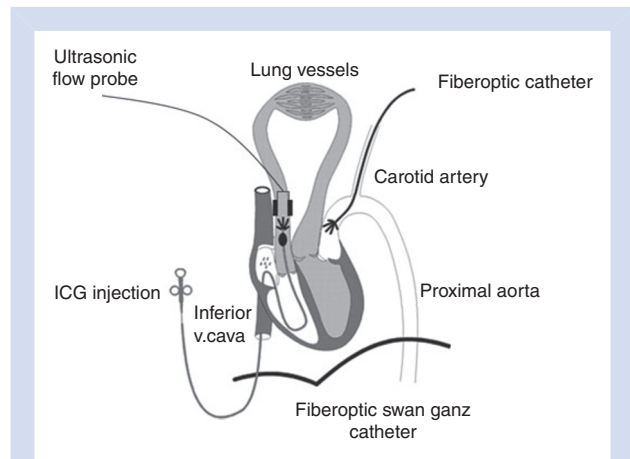


Fig 1 Illustration depicting the principles of the double indicator dilution technique. Indocyanine green (ICG) is injected into the right atrium. Two fiberoptic probes, placed in the pulmonary artery and ascending aorta, continuously measure ICG concentration. From the obtained dye dilution curves, mean transit times between injection site, pulmonary artery and aorta can be calculated to obtain ITBV and PBV.

guidelines for animal care (NIH publication nr 86-23, revised 1985). The dogs were raised and housed in single cages in the Animal Research Laboratory of the Heinrich-Heine University, Düsseldorf, Germany. Furthermore, the study was approved by the local District Governmental Animal Investigation Committee of North-Rhine Westphalia in Düsseldorf, Germany (Ref. 23.05-230-3-84/96). Five of the dogs used in this study were also used in another study published previously.¹⁵ For the experiments, the dogs were transferred to a special animal laboratory within the Department of Experimental Anaesthesiology of the University of Düsseldorf, Germany.

Several weeks before the actual experiments were performed, both carotid arteries were exteriorized in skin loops and an ultrasound transit-time flow probe (16–20 mm S-series with silicone shielded U-reflector, Transonic Systems, NY, USA) was implanted around the pulmonary artery (Fig. 1) through a left-sided thoracotomy, for continuous recording of pulmonary blood flow and subsequent continuous recording of cardiac output ('CO_{Transonic}'), as described in detail before.¹⁶ We introduced two catheters via the carotid arteries into the ascending aorta, one for blood sampling and measurement of arterial blood pressure and the other for insertion of a fiberoptic thermistor probe (4F, PulsioKath PV 2024, Pulsion Medical Systems, Munich, Germany) (Fig. 1). This probe continuously recorded intravascular indocyanine green (ICG) concentration and blood temperature. In addition, a 7F fiberoptic thermodilution pulmonary artery catheter (Arrow International, Reading, MA) was introduced (Fig. 1) via a dog's hindlimb under fluoroscopy before each experiment.

Measurements

Mean arterial blood pressure (MAP) and CVP (both measured using a Gould Statham pressure transducer P23 ID, Elk Grove, IL, USA, and adjusted to the level of the heart) were continuously recorded on an eight-channel polygraph (model RS 3800, Gould, Cleveland, OH) and simultaneously on a cassette data recorder (model XR-5000, TEAC®, Tokyo, Japan).

Transpulmonary dye dilution allows the determination of a volume of blood between a proximal dye injection site and a distal recording site. The dye distribution volume (i.e. the blood volume of

that compartment) is the product of flow and the mean transit time (mtt) of the dye between these sites. In this study, all blood volumes were determined as the product of $CO_{Transonic}$ and mtt of the dye indocyanine green (ICG).^{17, 18} ICG (0.2 mg kg⁻¹, 5 ml), was injected via the proximal port of the pulmonary artery catheter into the right atrium and the resulting thermo- and dye-dilution curves were recorded in the pulmonary artery and ascending aorta simultaneously with fiberoptic-probes connected to an optoelectronic device (COLD-System Z 021, Pulsion, Munich, Germany).

ITBV, representing the volume of blood between the right atrium and ascending aorta, was calculated as the product of $CO_{Transonic}$ and mtt of ICG between injection site of ICG (right atrium) and ascending aorta. PBV, which represents the volume of blood between the pulmonary and the aortic valve, was calculated as the product of $CO_{Transonic}$ and the mtt of ICG between the pulmonary artery and ascending aorta. The mtt of PBV was determined by deconvolution of the dye dilution curves, based on a pulmonary transport function as described in detail before.¹⁹

Finally, $V_{d\ circ}$ was calculated as the product of $CO_{Transonic}$ and mtt of ICG through the circulation (mtt_{circ}), (measured over a 30 min time period). Mtt_{circ} was obtained by fitting the aortic dye dilution curve to a recirculation model.²⁰ Transport of a dye through circulating blood volume is best defined by a two-compartmental model.¹⁹ This model essentially consists of a fast and slowly perfused compartment and hence, mtt_{circ} is the sum of the individual mtt through the fast (mtt_1) and slow (mtt_2) compartment, corrected by associated correction factors (R_1 and R_2 , respectively), to compensate for loss of tracer during passage (e.g. hepatic elimination). The product of $CO_{Transonic}$ with ($mtt_1 * R_1$) or ($mtt_2 * R_2$) yields the respective volumes of distribution of the fast (V_{d1}) and slow (V_{d2}) compartment.

To assess the influence of alterations in blood volume on the distribution of blood volumes, the ratios between PBV and $V_{d\ circ}$ ($PBV / V_{d\ circ}$) and ITBV and $V_{d\ circ}$ ($ITBV / V_{d\ circ}$) were calculated. In addition, the ratio between V_{d1} and V_{d2} was calculated as $(CO_{Transonic} * mtt_1 * R_1) / (CO_{Transonic} * mtt_2 * R_2)$, which is equal to $(mtt_1 * R_1) / (mtt_2 * R_2)$.

Experimental program

The animals were anaesthetized with pentobarbital (initial dose 20 mg kg⁻¹ followed by 4 mg kg⁻¹ h⁻¹). They were allowed to breathe room air (FiO_2 0.21) spontaneously to exclude effects of mechanical ventilation with positive pressures on the distribution of blood volume. After introduction of the catheters into the pulmonary artery and ascending aorta, we observed the dogs for 30 min to ensure stable haemodynamic conditions before we started data collection.

Blood volume was altered in both directions in a total of 68 steps of 150–450 ml (up to about 20% of estimated total blood volume), lasting 10 minutes each, by either (up to about 20% of estimated total blood volume) by either haemorrhage and subsequent retransfusion of the shed blood, or by infusion of colloid solution (hydroxyethyl starch (HES 200/0.5 6% solution, Fresenius Kabi GmbH, Bad Homburg, Germany). An intervention interval lasted for 25 min, 10 min for performing the intervention (blood withdrawal or infusion, respectively) and 15 min for establishing haemodynamic steady state conditions, after/at the end of which measurements were obtained. After seven to eight min, a duplicate measurement of PBV, ITBV and $V_{d\ circ}$ was performed and a mean value was used for subsequent calculation. In case the obtained measurement values showed a substantial difference of more than 20%, a third measurement was performed and a new mean value was calculated.

The alterations in blood volume were performed in a randomly assigned fashion in order to reduce the possibility of systematic bias. The researchers were not blinded to the type of intervention performed. Furthermore, all measurements were performed at the end of each intervention when all variables were in a steady state.

All experiments were carried out under standardized conditions, at a thermoneutral temperature for dogs, which is at 24° C. Body temperature was measured rectally and was kept within the physiologic range for dogs (i.e. 37.0–38.5°C) by application of warming blankets. Each dog was allowed to recover at least for one week between experiments. After completion of the experiments, the chronically instrumented dogs were maintained for the rest of their lives in the animal research facility of our institution, for use in other experiments until they died of natural causes.

Statistical analysis

Statistical analysis was performed using Microsoft Excel 2010 (Microsoft, Redmond, USA) and SPSS Statistics version 19.0 (SPSS Inc., Chicago, USA). Normal distribution of continuous data was assessed using the Kolmogorov-Smirnov test. $CO_{Transonic}$ values were normalized on body weight.^{21, 22} The correlation between blood volumes was displayed in a scatterplot and the coefficients of determination (R^2 values) were calculated together with 95% confidence intervals, after correcting for the repeated character of the study by removing the between-subject variation.²³

Haemodynamic variables were analysed in separate groups, based on the degree of alteration of blood volume [normovolaemia, a decrease/increase of 0–150 ml ('mild'), or 150–450 ml ('moderate')]. In order to account for between-subject variation and because the alterations of blood volume were performed in a randomly assigned order, regression analysis was performed using a linear mixed model. Using this model, the influence of blood volume alterations on the measured haemodynamic variables and blood volumes could be analysed for an overall effect between normovolaemia and hypo-/hypervolaemia. In addition, differences between the defined groups (as described above) were analysed using this model. All performed tests were two-sided and statistical significance was set at a P -value <0.05 after adjusting for multiple comparisons using the Bonferroni correction.

Results

A total of 90 measurements were obtained in 22 experiments in 6 dogs. Changes in $V_{d\ circ}$ ranged from –33% (hypovolaemia, $n=31$) to +31% (hypervolaemia, $n=28$). No adverse events occurred.

Heart rate increased significantly during moderate hypovolaemia, while CVP significantly decreased (Table 1). The increase in heart rate from mild to moderate hypovolaemia was also significant (Table 1). Arterial PCO_2 remained stable throughout the interventions and was 5.8 (0.2) kPa for all data points.

During mild hypervolaemia (0–150 ml), none of the investigated haemodynamic variables were significantly different compared with normovolaemia, while during moderate hypervolaemia (150–450 ml), $CO_{Transonic}$, MAP and CVP significantly increased (Table 1). MAP was also significantly higher during moderate than during mild hypervolaemia (Table 1).

$V_{d\ circ}$, PBV and ITBV during hypo- and hypervolaemia

The scatterplot in Fig. 2 shows the correlation between changes in blood volume and values of $V_{d\ circ}$, PBV and ITBV for all measured data points, together with the resultant slope of the linear

Table 1 Haemodynamic changes in response to alterations in blood volume. Data are presented as mean (95% confidence interval of the mean; SD) and median (range). $V_{d \text{ circ}}$: Circulating blood volume; MAP: Mean Arterial Pressure; CVP: Central Venous Pressure; $CO_{\text{Transonic}}$: Cardiac Output derived from Transonic Flow Probe; PBV: Pulmonary Blood Volume; ITBV: Intrathoracic Blood Volume. Data were analysed separately for hypo- and hypervolaemia using a linear mixed model analysis and were tested against baseline values during normovolaemia. ^a $P < 0.05$ for the overall effect for hypovolaemia (vs normovolaemia), as determined by the mixed model. ^b $P < 0.05$ for the overall effect for hypervolaemia (vs normovolaemia), as determined by the mixed model. * $P < 0.05$, for the comparison of mild or moderate hypo- or hypervolaemia vs the baseline value during normovolaemia. [†] $P < 0.05$ for the comparison of moderate vs mild hypo- or hypervolaemia

Normovolaemia (n=31)		Hypovolaemia		Hypervolaemia	
		Mild (n=15)	Moderate (n=16)	Mild (n=10)	Moderate (n=18)
Relative volume status (ml kg ⁻¹)		-5 (-4/-5; 2)	-11 (-9/-12; 3)	4 (2-5; 2)	11 (9-12; 3)
		-5 (-1/-6)	-11 (-6/-17)	4 (1-6)	10 (6-20)
Response of conventional haemodynamic variables					
MAP (mm Hg) ^b	86 (83-90; 10)	83 (76-91; 13)	89 (83-95; 11)	81 (70-92; 15)	93 (86-101; 15) [†]
	88 (63-106)	85 (58-103)	93 (57-98)	85 (59-105)	92 (65-117)
Heart rate (bpm) ^a	94 (88-101; 17)	91 (83-99; 15)	110 (103-117; 13) [†]	84 (69-99; 21)	85 (76-94; 19)
	88 (67-136)	89 (69-129)	108 (91-133)	83 (49-119)	87 (47-125)
CVP (mm Hg) ^{a,b}	2 (1-2; 1)	2 (1-3; 1)	1 (0-1; 1)*	3 (2-5; 2)	4 (3-6; 3)*
	2 (-1-4)	1 (0-4)	1 (-1-3)	3 (0-7)	3 (1-12)
$CO_{\text{Transonic}}$ (ml min ⁻¹ kg ⁻¹) ^{a,b}	94 (88-99; 16)	79 (71-87; 15)*	79 (73-86; 12)*	92 (82-101; 13)	108 (96-120; 25)*
	95 (60-124)	77 (52-101)	78 (62-98)	92 (68-107)	106 (62-152)
Response of measured blood volumes and their ratios:					
Change in $V_{d \text{ circ}}$ (%)		-9 (-7/-11; 3)	-21 (-19/-24; 5)	8 (5-11; 5)	21 (18-23; 5)
		-11 (-2/-14)	-22 (-14/-33)	8 (2-14)	21 (11-13)
$V_{d \text{ circ}}$ (ml kg ⁻¹) ^{a,b}	50.9 (47.8-53.9; 8.1)	47.4 (43.9-50.8; 6.2)	41.6 (39.6-43.7; 3.9) [†]	52.0 (48.7-55.3; 4.6)	57.7 (53.4-62.0; 8.7) [†]
	51.0 (35.0-66.0)	49.0 (33.0-56.0)	42.0 (34.0-48.0)	51.5 (44.0-57.0)	58.0 (41.0-70.0)
PBV (ml kg ⁻¹) ^{a,b}	10.4 (9.7-11.0; 1.7)	9.5 (8.8-10.2; 1.2)*	9.1 (8.6-9.6; 0.9) [†]	10.4 (9.4-11.4; 1.3)	11.9 (11.0-12.9; 1.9) [†]
	10.0 (7.0-14.0)	10.0 (7.0-11.0)	9.0 (8.0-11.0)	10.5 (8.0-13.0)	12.0 (8.0-15.0)
ITBV (ml kg ⁻¹) ^{a,b}	16.0 (4.9-16.9; 2.5)	14.4 (13.4-15.4; 1.9)*	13.3 (12.7-13.9; 1.1) [†]	16.2 (14.9-17.4; 1.8)	18.8 (17.5-20.2; 2.7) [†]
	16.0 (11.0-22.0)	15.0 (11.0-17.0)	13.0 (12.0-16.0)	17.0 (13.0-19.0)	19.5 (13.0-22.0)
PBV/ $V_{d \text{ circ}}$ ratio	0.20 (0.19-0.21; 0.02)	0.20 (0.19-0.21; 0.02)	0.22 (0.21-0.23; 0.02)	0.20 (0.18-0.21; 0.01)	0.21 (0.19-0.21; 0.02)
	0.20 (0.17-0.23)	0.20 (0.16-0.24)	0.22 (0.19-0.24)	0.20 (0.18-0.23)	0.20 (0.18-0.25)
ITBV/ $V_{d \text{ circ}}$ ratio ^b	0.31 (0.30-0.32; 0.02)	0.31 (0.29-0.33; 0.04)	0.32 (0.31-0.33; 0.02)	0.31 (0.29-0.33; 0.03)	0.33 (0.32-0.34; 0.02)*
	0.31 (0.27-0.34)	0.31 (0.23-0.36)	0.31 (0.27-0.35)	0.31 (0.26-0.35)	0.33 (0.29-0.38)

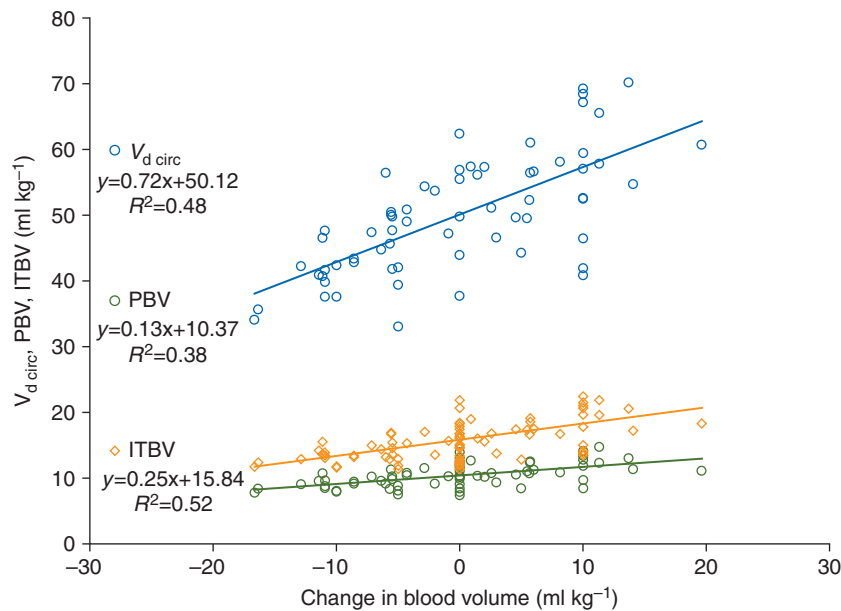


Fig 2 Scatterplot of change in blood volume vs corresponding values of $V_{d \text{ circ}}$, PBV and ITBV. The linear regression lines, together with the respective coefficients of determination (R^2) are shown.

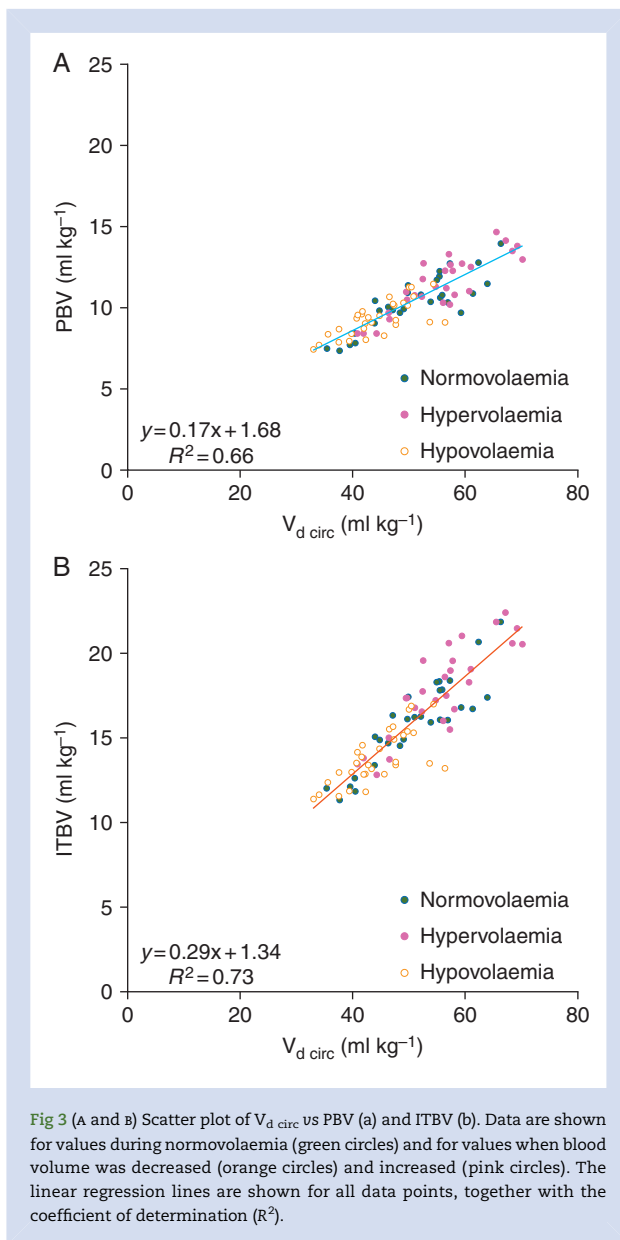


Fig 3 (A and B) Scatter plot of $V_{d \text{ circ}}$ vs PBV (a) and ITBV (b). Data are shown for values during normovolaemia (green circles) and for values when blood volume was decreased (orange circles) and increased (pink circles). The linear regression lines are shown for all data points, together with the coefficient of determination (R^2).

regression curve. The correlation between these variables was significant ($P < 0.05$) with associated R^2 values of 0.48 (95% Confidence Interval (CI) of 0.33–0.63) for $V_{d \text{ circ}}$, 0.38 (CI 0.23–0.53) for PBV, and 0.52 (CI 0.38–0.66) for ITBV.

The correlation between $V_{d \text{ circ}}$ and PBV for data points during normo-, hypo- and hypervolaemia is shown in Fig. 3A. Overall correlation was significant ($P < 0.05$) with an R^2 value of 0.66 (CI: 0.55–0.77). In addition, the correlation between $V_{d \text{ circ}}$ and ITBV (Fig. 3B) for all data points was significant ($P < 0.05$) with an associated R^2 value of 0.73 (CI 0.64–0.82).

During mild hypovolaemia, PBV and ITBV significantly decreased compared with normovolaemia. $V_{d \text{ circ}}$ also tended to decrease, although statistical significance was not reached ($P = 0.055$; Table 1). During moderate hypovolaemia, all blood volumes were significantly lower compared with both normovolaemia and mild hypovolaemia (Table 1). No changes in blood volumes were observed during mild hypervolaemia, while during moderate hypervolaemia all blood volumes increased

significantly compared with both normovolaemia and mild hypervolaemia (Table 1).

Blood volume changes and its ratio

The ratio between PBV and $V_{d \text{ circ}}$ (PBV/ $V_{d \text{ circ}}$ ratio as an assessment of the (re)distribution of blood between intra- and extra-thoracic compartments) remained constant for all gradations of both hypo- and hypervolaemia. The same applies to the ratio between ITBV and $V_{d \text{ circ}}$ (ITBV/ $V_{d \text{ circ}}$ ratio), except for the data points obtained during moderate hypervolaemia, where the ITBV/ $V_{d \text{ circ}}$ ratio was slightly increased. However, the correlation between blood volume changes and the PBV/ $V_{d \text{ circ}}$ and ITBV/ $V_{d \text{ circ}}$ ratio was not significant (Fig. 4), indicating a constant ratio (R^2 0.04 and 0.05, respectively).

$V_{d \text{ circ}}$ and the difference between ITBV and PBV correlated linearly with an R^2 value of 0.60 (95% CI: 0.48–0.72; $P < 0.05$) (Fig. 5).

The ratio between the fast and slow compartment of circulating blood volume (the $(mtt_1 \cdot R_1)/(mtt_2 \cdot R_2)$ ratio) was not correlated with alterations of blood volume ($R = 0.03$; $P = 0.8$, data not shown).

In Supplementary data, Figure S2 (online-only), the distribution between R_1 , R_2 and mtt_1 , mtt_2 respectively, is shown for data points obtained during normo-, hypo- and hypervolaemia. In this figure, it can be seen that there is no substantial shift of the relationship R_1 vs mtt_1 or R_2 vs mtt_2 during any of the alterations in volume status.

Discussion

In this study in anaesthetized spontaneously breathing dogs, hypo- and hypervolaemia were found to induce linear changes in central blood volumes (PBV and ITBV) and $V_{d \text{ circ}}$ for alterations up to $(20) \text{ ml kg}^{-1}$ of $V_{D \text{ circ}}$. The ratio between the central blood volumes and $V_{d \text{ circ}}$ was maintained, suggesting that under the studied circumstances, blood volume distribution between central (intrathoracic) compartments and the peripheral (extrathoracic) compartment is maintained. Clinically, it could be suggested that, during anaesthesia, (small) blood volume changes can affect cardiac preload, stressing the importance of fluid homeostasis for preserving venous return and thus cardiac output.

An important consideration in this study is the reliability of the volumetric measurements. During normovolaemia, mean $V_{d \text{ circ}}$ was about 51 ml kg^{-1} , which is only 60% of the expected total blood volume in dogs (80 ml kg^{-1}).²⁴ Although dye dilution derived $V_{d \text{ circ}}$ has been shown to be reliable compared with reference techniques (i.e. Evans blue), it underestimates total blood volume by approximately 40%,^{7 19 20} most likely because of the rather short measurement time (5 min), resulting in incomplete dye mixing in slowly perfused body compartments. $V_{d \text{ circ}}$ therefore does not include these compartments and reflects circulating instead of total blood volume. Nevertheless, $V_{d \text{ circ}}$ correlated moderately with blood volume alterations, suggesting that the dye dilution permits accurate measurement of $V_{d \text{ circ}}$ both during hypo- and hypervolaemia, although this finding also demonstrates that not all volume alterations are solely reflected in corresponding $V_{d \text{ circ}}$ values (see further).

PBV and ITBV were previously demonstrated to be accurately measured by transpulmonary dye dilution, both in humans^{8 19} and in animals.²⁵ The close correlation of $V_{d \text{ circ}}$ with both PBV and ITBV (Fig. 3), additionally supports that this technique allows measuring changes in these variables during hypo- and hypervolaemia, even with a low intra-individual variability (7%, data not shown).

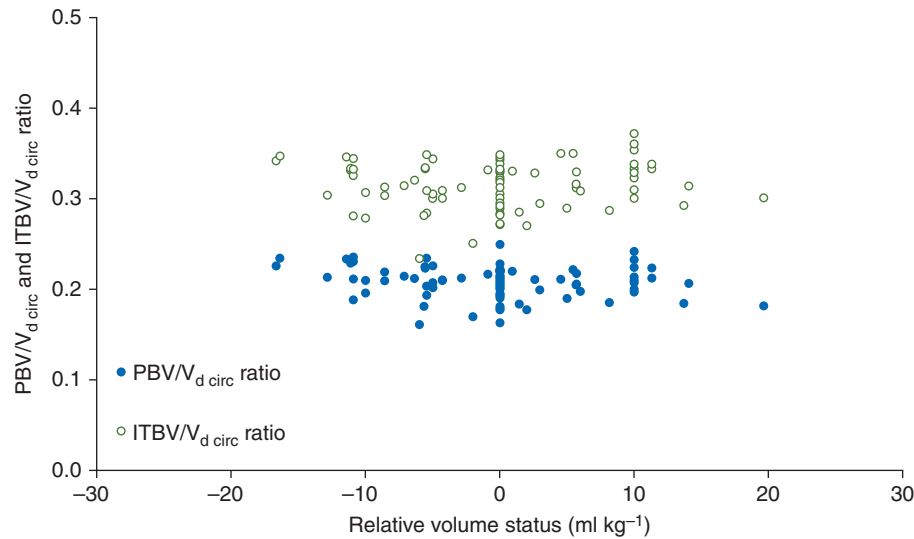


Fig 4 Scatter plot of the relative volume status vs the $PBV/V_{d\text{ circ}}$ and $ITBV/V_{d\text{ circ}}$ ratio for all data points.

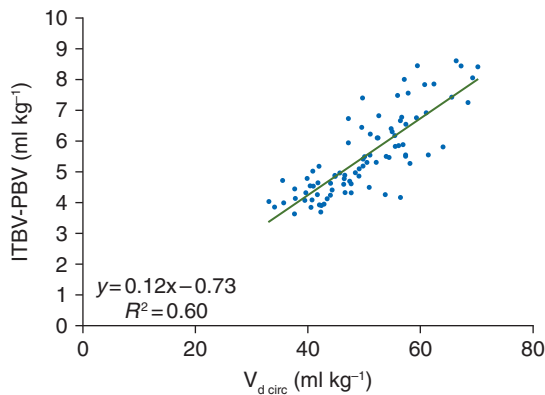


Fig 5 Correlation between $V_{d\text{ circ}}$ and the difference between ITBV and PBV. The linear regression line is shown together with the coefficient of determination (R^2).

It is well known that multiple factors influence blood volume distribution between intra- and extrathoracic compartments, such as the induction of anaesthesia or presence of severe emphysema.^{8,26–28} Our observation that both PBV and ITBV decrease during hypovolaemia is similar to previous studies in swine²⁹ and humans,³⁰ though despite extensive research,³¹ the effects of intravascular hypo- and hypervolaemia on the actual blood volume distribution have not yet been fully explored in animal or human studies. Unfortunately, studying blood volume distribution in humans using our experimental setup, would apparently not be suitable. The application of 'lower body negative pressure' in humans allows simulating hypovolaemia^{9,10} yet this setup influences blood volume distribution by itself and impedes assessing blood volume distribution.

The observed maintained ratio between $V_{d\text{ circ}}$ and PBV/ITBV during all grades of hypovolaemia as a result of haemorrhage,

suggests that blood is lost from the circulation as a whole, not only from the non-central, extrathoracic compartments. This observation is in accordance with clinical findings that hypovolaemia reduces cardiac preload, which is directly recruited from the central blood volumes, and induces pulmonary vasoconstriction.³² As already discussed, $V_{d\text{ circ}}$ represents about 60% of total blood volume. The observed slope of $V_{d\text{ circ}}$ for changes in blood volume was 0.72 (Fig. 2), meaning that if blood volume were decreased from normovolaemia $V_{d\text{ circ}}$ 50 ml kg⁻¹, total blood volume 80 ml kg⁻¹ by for example 20 ml kg⁻¹, $V_{d\text{ circ}}$ would be $0.72 \times 20 = 36$ ml kg⁻¹. This example shows that the percentage change between $V_{d\text{ circ}}$ and total blood volume is constant, as a $V_{d\text{ circ}}$ value of 36 ml kg⁻¹ equals 60% of (80–20) ml kg⁻¹. The same calculation could also be applied to PBV or ITBV and demonstrates that there is no change between the central compartment (PBV, ITBV), circulating compartment and total blood compartment.

Interestingly, our findings also suggest that blood volume rearrangement from the slowly to the more rapidly perfused compartment, does not occur during general anaesthesia in the investigated range of blood volume alterations (online-only Supplementary data, Fig. S2).

As explained, all measurements were performed under deep sedation. The resulting sympatholysis¹³ probably blunts the sympathetic venous (and arterial) vasoconstriction. The importance of this reflex in preserving central blood volume during lower body negative pressure induced acute hypovolaemia, was demonstrated previously^{9,10} in awake patients with intact reflexes. The blunted 'centralization' of the circulation in case of hypovolaemia during general anaesthesia, was also demonstrated previously in dogs,¹² as a smaller amount of blood loss caused hypotension during general anaesthesia compared with awake circumstances. Unfortunately, none of these studies focused on the actual distribution of blood volume, but nevertheless they support our observation of a blunted 'centralization' reflex in case of hypovolaemia during general anaesthesia.

Extrapolation of the current results to a clinical setting might imply that in anaesthetized subjects, cardiac preload could become reduced at a relatively small reduction in blood volume.

Therefore, early fluid resuscitation might be required for preserving preload and maintaining cardiac function on the optimal part of the Frank-Starling curve for optimization of cardiac output, stressing the value of early goal-directed fluid therapy. While volumetric variables are infrequently measured clinically, ITBV has the advantage over PBV that it does not require pulmonary artery catheterization and can be measured less invasively. Also, ITBV has been shown equivalent with echocardiography-derived end diastolic-area values, reflecting preload.^{18 19 33} Therefore, the current results might indicate that in selected patients, advanced haemodynamic monitoring could be used to detect and reverse (even subtle) haemodynamic derangements.

In the opposite direction,—induced hypervolaemia,—we observed that PBV and ITBV only increased significantly in case of moderate hypervolaemia. Although one might argue that the resulting effect size of the observed differences between mild and moderate hypervolaemia was insufficient to demonstrate an increase in PBV and ITBV in mild hypervolaemia, it might also indicate that the cardiovascular system is able to adequately compensate for the increased filling during mild hypervolaemia, probably by prepulmonary venous dilatation. This hypothesis is supported by the observation that for moderate hypervolaemia, the ITBV/ $V_{d\text{ circ}}$ ratio increased slightly (Table 1), which was not true for the PBV/ $V_{d\text{ circ}}$ ratio. Essentially, the difference between ITBV and PBV reflects the prepulmonary blood volume (i.e. the volumes of the right atrium and right ventricle). Although PBV and ITBV were well correlated, we found a slope between the difference of ITBV - PBV and $V_{d\text{ circ}}$ (Fig. 5) which indicates that prepulmonary blood volume is influenced by intravascular volume shifts. In a recent study in humans, a similar volume-dependent difference between PBV and ITBV was found.³⁴

Study limitations

Firstly measurements were obtained in dogs and therefore data cannot be transposed to humans directly. Nevertheless, investigating haemodynamic physiology in middle-large mammals is very common. Furthermore, it allowed the randomization of the blood volume alterations in order to reduce the possibility of systemic bias, while keeping other potential influences standardized. Nevertheless, the randomization might have also added some degree of 'noise' to the data (e.g. hypovolaemia after normovolaemia vs hypovolaemia after hypervolaemia could have elicited (subtly) different physiologic responses).

Secondly, while the anaesthetized dogs were allowed to breathe spontaneously, to prevent a reduction in central blood volumes associated with positive pressure mechanical ventilation,^{8 26} the current findings might be less directly applicable to ventilated patients. Yet the findings might accurately reflect physiologic changes in non-ventilated patients (e.g. in trauma patients or in surgical patients under regional anaesthesia) with absolute hypo- or hypervolaemia. In addition, spontaneous respiration might induce haemodynamic variability of the measured variables.³⁵ Therefore, ice-cold ICG injections were spread equally over the respiratory cycle.

In addition, dye dilution derived variables such as ITBV bear the risk of 'mathematical coupling' with cardiac output. Although previously it has been shown that such mathematical coupling is unlikely,^{36 37} we chose to derive cardiac output independently using the ultrasound flow probe.¹⁶ Also, as cardiac output was the common factor in all blood volumes, these variables were also directly mathematically uncoupled.

Furthermore, splenic vasodilation or -constriction in dogs could substantially alter blood volume distribution in case of

hypo- or hypervolaemia.³⁸ However because of the anaesthesia related sympatholysis, the influence of the spleen on blood volume distribution was rendered negligible.

Finally, blood volume was altered $\pm 20\%$ $V_{d\text{ circ}}$. Therefore, current data cannot be extrapolated to more severe hypo- or hypervolaemic conditions. Especially for more severe hypovolaemia, blood volume distribution could become altered by additional compensatory cardiovascular control mechanisms for maintaining cardiac preload. As we did not intend to induce irreversible shock in our chronically instrumented dogs we restricted blood volume alterations up to ± 450 ml. Yet, such alterations up to 20% are relatively common in patients in anaesthesia and critical care setting, reflecting the clinical importance of the current findings.

Conclusions

In conclusion, the current findings suggest that in anaesthetized spontaneously breathing dogs in which blood volume was altered to induce moderate hypo- or hypervolaemia, the cardiovascular system maintains a constant distribution between central and circulating blood volume.

Authors' contributions

Study design/planning: T.W.L.S., J.K.G.W. Study conduct: T.W.L.S., J.K.G.W. Data analysis: J.J.V., J.K.G.W. Writing paper: J.J.V., T.W.L.S., S.A.L., A.H., J.K.G.W. Revising paper: all authors

Supplementary material

Supplementary material is available at *British Journal of Anaesthesia* online.

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Declaration of interest

None declared.

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